Epilepsy & Behavior 98 (2019) 101–109

Contents lists available at ScienceDirect

Epilepsy & Behavior

journal homepage: www.elsevier.com/locate/yebeh

Assessment of genes involved in behavior, learning, memory, and synaptic plasticity following status epilepticus in rats

Mehmet Fatih Göl [a,*], Füsun Ferda Erdoğan [b], Kezban Korkmaz Bayramov [c], Ecmel Mehmetbeyoğlu [c], Yusuf Özkul [d]

[a] Department of Neurology, Kayseri City Hospital, Kayseri, Turkey
[b] Department of Neurology, Erciyes University Faculty of Medicine, Kayseri, Turkey
[c] Erciyes University Genome and Stem Cell Center (GENKOK), Kayseri, Turkey
[d] Department of Medical Genetics, Erciyes University Faculty of Medicine, Kayseri, Turkey

ARTICLE INFO

Article history:
Received 21 March 2019
Revised 9 June 2019
Accepted 11 June 2019
Available online 19 July 2019

Keywords:
Status epilepticus
Immature rat
Cognition and behavior
Synaptic plasticity gene expression

ABSTRACT

Objective: In this study, it was aimed to evaluate cognitive and behavioral changes after status epilepticus (SE) induced by pentylenetetrazole in immature rats via Morris water maze and open-field area tests and to assess alterations in expression of 84 key genes involved in synaptic plasticity after SE.
Method: The study was conducted on 30 immature rats (12-days old). The rats were assigned into groups as control and experiment (SE) groups. The SE was induced by pentylenetetrazole in 12-days old rats. In addition, experiment group was divided into two groups as mature (n = 8) and immature SE (n = 8) subgroups. Again, the control group was divided into two groups as mature (n = 7) and immature control (n = 7) subgroups. Hippocampal tissue samples were prepared, and expression of 84 key genes involved in synaptic plasticity was assessed in Genome and Stem Cell Center of Erciyes University before behavioral tests in immature rats (22-days old) and after open-filed area and Morris water maze tests in mature rats (72-days old) in both experiment and control groups.
Results: No significant difference was detected in behavioral tests assessing spatial memory and learning among groups. Significant differences were detected, ARC (activity-regulated cytoskeleton-associated protein), BDNF (brain-derived neurotrophic factor), MAPK1 (mitogen-activated protein kinase 1), NR4A1 (nuclear receptor subfamily 4 group A member 1), PPP3CA (protein phosphatase 3 catalytic subunit alpha), RGS2 (regulator of G protein signaling 2), and TNF (tumor necrosis factor) gene expressions between control and experiment groups in immature rats whereas in ADCY8 (adenylate cyclase 8), BDNF (brain-derived neurotrophic factor), EGR4 (early growth response 4), and KIF17 (kinesin family member 17) gene expressions between control and experiment groups in mature rats.
Discussion: In this study, differences detected in gene expressions of synaptic plasticity after SE indicate in which steps of synaptic plasticity may be problematic in epileptogenesis. The gene expressions in this study may be considered as potential biomarkers; however, epileptogenesis is a dynamic process and cannot be explained through a single mechanism. Future studies on epileptogenesis and studies specifically designed to evaluate genes detected in our study will further elucidate synaptic plasticity in epilepsy and epileptogenesis.

1. Introduction

Status epilepticus (SE) is defined as a single epileptic seizure or cluster of seizures without return of consciousness lasting more than 30 min [1]. There are ongoing attempts to clarify underlying mechanisms for prolonged and differing characteristics of SE compared to brief seizures. It has been found that hippocampus is continuously active during SE [2].

Epileptogenesis is defined as events that occur before first seizure, predispose epileptic brain to spontaneous recurrent seizures, enhance seizure severity, and make epilepsy resistant to treatment [3]. Epileptogenesis has numerous cellular mechanisms including cell loss, gliosis, increased expression of intermediate-early genes (c-fos, c-jun), increased growth factors, neurogenesis, synaptogenesis, altered glutamate and GABA signaling, inflammatory mediators, voltage-gated ion channel changes, and excitotoxic antibodies among others [4].

* Corresponding author at: Department of Neurology, Kayseri City Hospital, 38080 Kayseri, Turkey.
E-mail address: m-fatih-gol@hotmail.com (M.F. Göl).

https://doi.org/10.1016/j.yebeh.2019.06.023
1525-5050/

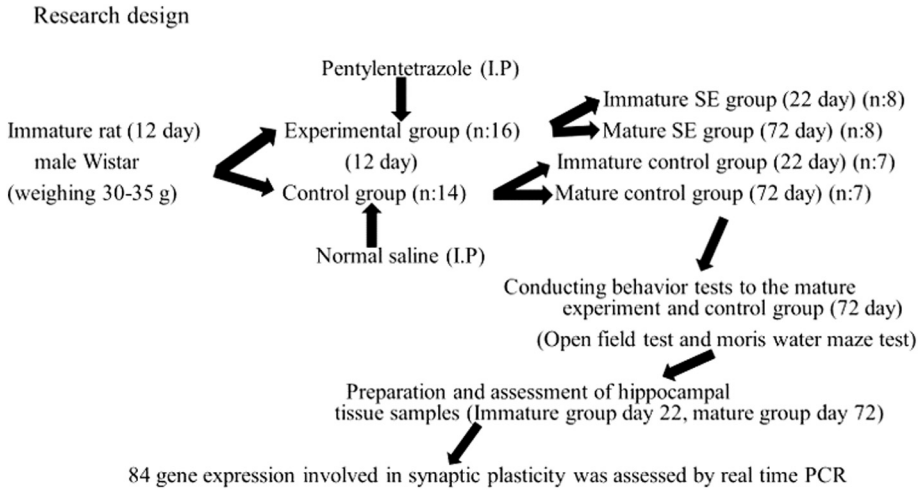


Fig. 1. Research design. Research plan is shown in the figure. I.P: intraperitoneal.

The plasticity is pivotal in emotional and cognitive behaviors, learning, and memory, which have important roles in the formation of neuronal network in the brain [5]. Synapse formation is mainly controlled by genetics; however, only minority of the genes regulating synapse formation is known [6].

The rapid and strong stimulation of presynaptic neurons generates action potential in postsynaptic neuron. Over time, these synapses become more susceptible, and stimulus is increasingly transmitted to postsynaptic region. The prolonged enhancement in synaptic transmission is termed as long-term potentiation (LTP). The slow and weak stimulation of neurons also leads to changes in synapses, resulting in decrease in transmission of stimulus to postsynaptic region. This is termed as long-term depression (LTD). The LTP and LTD have introduced novel ideas about molecular mechanisms of memory. Ongoing LTP and LTD for many hours can also change transmitter release and receptors on neuronal surface. The longevity of LTP and LTD requires genetic transcription and translation [7–12]. It was shown that LTP does not only enhance synaptic connections but also promote new neuron formation in hippocampus during learning process [13]. It was found that LTP induces younger neurons more readily and that younger neurons have higher amplitude than mature neurons; thus, these new neurons enhance synaptic plasticity in hippocampus [14]. It was also found that brain-derived neurotrophic factor (BDNF) at a certain threshold is required for LTP formation and that excessive BDN (brain-derived neurotrophic) inhibits LTD [15].

In the brain, neuronal gene expression is a dynamic process because of neuronal activity. The expression of immediate-early response genes (IEG) is selectively upregulated in neuron subgroups at brain regions related to learning and memory formations. Optogenetic and pharmacogenetic studies revealed that IEG-positive neurons encode and store information required for recall, suggesting that they may have roles in the creation of memory traces [16].

Many extrinsic and intrinsic inputs are needed for maturation of brain regions related to cognitive functions. Seizure activity can hamper this process by suppressing these regions and can cause atypical lateralization of cerebral functions [17]. Understanding molecular pathways of neuronal damage induced by seizures will reduce damage and cognitive destruction in epilepsy and will have implications on epileptogenesis occurring following brain injury [18]. As epileptogenesis studies are difficult and unethical in human, animal models of epileptogenesis are warranted [19].

In this study, it was aimed to assess alterations in expression of 84 key genes involved in synaptic plasticity after SE induced by pentylenetetrazole (PTZ) in immature rats, and to evaluate cognitive

Table 1
Functional gene groups of synaptic plasticity.

Immediate-early response genes (IEGs)	Arc[a], Bdnf[a], Cebpb, Cebpd, Creb1, Crem, Egr1, Egr2, Egr3, Egr4[a], Fos, Homer1, Jun, Junb, Klf10, Mmp9 (gelatinase B), Nfkb1, Nfkbib (Trip9), Ngf, Nptx2, Nr4a1[a], Ntf3, Pcdh8, Pim1, Plat (tPA), Rela, Rgs2[a], Rheb, Srf, Tnf[a]
Late response genes	Inhba, Synpo
Long-term potentiation (LTP)	Adcy1, Adcy8[a], Bdnf[a], Camk2a, Camk2g, Cdh2 (N-cadherin), Cnr1, Gabra5, Gnai1, Gria1, Gria2, Grin1, Grin2a, Grin2b, Grin2c, Grin2d, Mapk1[a], Mmp9 (gelatinase B), Ntf4, Ntrk2, Plcg1, Ppp1ca, Ppp1cc, Ppp3ca[a], Prkca, Prkcg, Rab3a, Ywhaq (14-3-3)
Long-term depression (LTD)	Gnai1, Gria1, Gria2, Gria3, Gria4, Grip1, Grm1, Grm2, Igf1, Mapk1[a], Nos1, Ngfr, Pick1, Plat (tPA), Ppp1ca, Ppp1cc, Ppp1r14a (Cpi-17), Ppp2ca, Ppp3ca[a], Prkca, Prkg1
Cell adhesion	Adam10, Cdh2 (N-cadherin), Grin2a, Grin2b, Ncam1, Pcdh8, Ppp2ca, Reln, Tnf[a]
Extracellular matrix and proteolytic processing	Adam10, Mmp9 (gelatinase B), Plat (tPA), Reln, Timp1
CREB cofactors	Akt1, Camk2g, Grin1, Grin2a, Grin2b, Grin2c, Grin2d, Mapk1[a], (Erk2), Ppp1ca, Ppp1cc
Neuronal receptors	Ephb2, Gabra5, Gria1, Gria2, Gria3, Gria4, Grin1, Grin2a, Grin2b, Grin2c, Grin2d, Grm1, Grm2, Grm3, Grm4, Grm5, Grm7, Grm8, Ntrk2
Postsynaptic density	Adam10, Arc[a], Dlg4 (Psd95), Gria1, Gria3, Gria4, Grin1, Grin2a, Grin2b, Grin2c, Grm1, Grm3, Homer1, Pick1, Synpo
Others	Kif17[a], Sirt1

Arc: activity-regulated cytoskeleton-associated protein, Adcy8: adenylate cyclase 8, Bdnf: brain-derived neurotrophic factor, Egr4: early growth response 4, Kif17: kinesin family member 17, Mapk1: mitogen-activated protein kinase 1, Nr4a1: nuclear receptor subfamily 4 group A member 1, Ppp3ca: protein phosphatase 3 catalytic subunit alpha, Rgs2: regulator of G protein signaling 2, Tnf: tumor necrosis factor.
[a] There was a statistically significant difference between groups.

Table 2
Comparison of rats' weight (gram).

Groups	n (sample size)	Weight (gram)	p value
Experimental group (12 day)	16	33 ± 2	>0.05
Control group (12 day)	14	32 ± 2	
Immature SE group (22 day)	8	50 ± 4	>0.05
Immature control group (22 day)	7	52 ± 4	
Mature SE group (72 day)	8	220 ± 8	>0.05
Mature control group (72 day)	7	222 ± 10	

Comparison of rat weight (gram). The weight of the rats was measured before injection and before the hippocampal tissue samples were prepared. Data are presented as mean ± standard error of the mean.

Table 3
Open-field test parameters of control and experimental groups.

Groups	Number of defecation	Number of rearing	Number of freezing	Number of grooming	Number of line passes (number/5 min)	Time spent in the center (s)	Time spent in the periphery (s)
Mature SE	3.6	6.2	1(0–8)	5.4	20 (15–25)	4 (3–21)	296 (279–297)
Control	2.6	3	1(0–3)	4.4	15 (12–26)	2 (1–20)	298 (280–299)
p	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05

Values referred as median (1st–3rd quartiles).

and behavioral changes via using Morris water maze (MWM) and open-field area tests after SE.

2. Material and methods

2.1. Animals

The study protocol was approved by Institutional Ethics Committee on Animal Experiments of Erciyes University (HADYEK; approval#: 16/022; 13.01.2016). The study was conducted on 30 male Wistar rats (12-days old; weighing 30–35 g) purchased from Hakan Çetinsaya Experimental and Clinical Research Center (DEKAM), Erciyes University.

All rats were kept in an environment with temperature of 22–24 °C under 12/12 light/dark cycle with food and tap water provided ad libitum. All experiments were performed at the same hour in order to prevent influence of circadian rhythm changes. The rats were assigned into groups as the control and the experiment groups. Then, the experiment group was divided into two groups as mature (n = 8) and immature SE (n = 8) subgroups. Again, the control group was divided into two groups as mature (n = 7) and immature control (n = 7) subgroups (Fig. 1).

Table 4a
Comparison of time latency to reach the platform in Morris water maze test.

Groups	Day 1	Day 2	Day 3	Day 4	p value
Mature SE group	55.1 ± 2.2	42.0 ± 4.2	22.3 ± 3.6	18.1 ± 4.2	<0.01
Mature control group	50.4 ± 4.1	37.8 ± 6.4	21.1 ± 4.1	18.0 ± 3.6	<0.01
p value	>0.05	>0.05	>0.05	>0.05	

Comparison of time latency to reach the platform in Morris water maze test. Data are presented as mean ± standard error of the mean.

Table 4b
Comparison of swimming speed in the test in Morris water maze test (cm/s).

Groups	Day 1	Day 2	Day 3	Day 4	p value
Mature SE group	25.56 ± 5.38	26.79 ± 4.45	28.60 ± 4.40	31.74 ± 4.42	<0.01
Mature control group	25.93 ± 4.06	26.18 ± 4.16	28.46 ± 4.24	30.61 ± 4.32	<0.01
p value	>0.05	>0.05	>0.05	>0.05	

Comparison of swimming speed in the test in Morris water maze test (cm/s). Data are presented as mean ± standard error of the mean.

Table 4c
Comparison of traveled distance to reach the platform in Morris water maze test.

Groups	Day 1	Day 2	Day 3	Day 4	p value
Mature SE group	726.26 ± 246.21	516.32 ± 240.44	470.63 ± 238.46	458.58 ± 236.86	<0.01
Mature control group	756.64 ± 241.41	526.16 ± 238.38	468.88 ± 237.68	440.62 ± 237.52	<0.01
p value	>0.05	>0.05	>0.05	>0.05	

Comparison of traveled distance to reach the platform in Morris water maze test. Data are presented as mean ± standard error of the mean.

Table 4d
The results of the time spent in target quadrant, 24 h after the last learning secession (%).

Groups	Time spent in target quadrant
Mature SE group	29.44 ± 4.24
Mature control group	29.52 ± 4.60
p value	>0.05

The platform was removed, and the time spent in target quadrant was compared. Data are shown as mean ± standard error of the mean.

2.2. Seizure model

To induce SE, PTZ was injected to 12-day-old rats in the experiment group while normal saline (NS) was given to the control group rats via intraperitoneal route. In order to create PTZ-induced animal model of SE, PTZ was given at a dose of 35 mg/kg initially, and repeated doses of 10 mg/kg were administered until SE development. The time interval

Table 5
Effect of status epilepticus on synaptic plasticity gene expression in immature rats.

Gene symbol	mRNA gene expression		p
	Control	Immature SE	
Arc	0.015913	0.009947	**0.03914**
Bndf	0.02398	0.034548	**0.017998**
Mapk1	0.758559	0.874936	**0.035638**
Nr4a1	0.035558	0.0258	**0.026212**
Ppp3ca	1.785506	2.413288	**0.014824**
Rsg2	0.038243	0.045705	**0.028457**
Tnf	0.000338	0.000201	**0.032034**

Median value of the seven gene expressions between the experimental and control groups in immature rats.
Statistically significant p values are shown in bold.

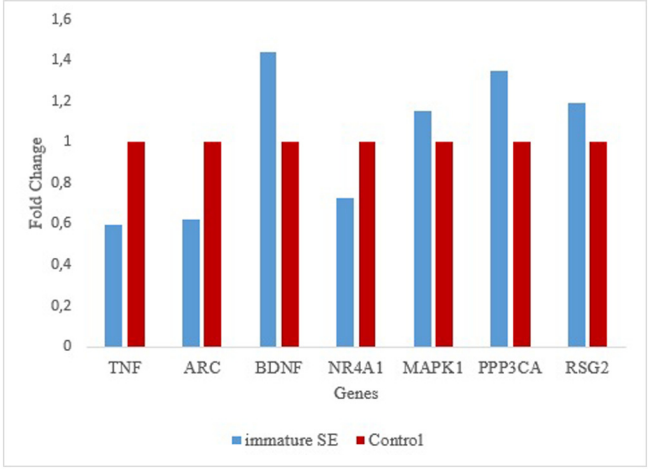


Fig. 2. Fold changes in immature SE. Fold changes of the seven gene expressions between the experimental and control groups in immature rats.

Table 6
Effect of status epilepticus on synaptic plasticity gene expression in mature rats.

Gene symbol	mRNA gene expression		p
	Control	Mature SE	
Adcy8	0.011559	0.024744	**0.01252**
Bdnf	0.018246	0.034423	**0.02911**
Egr4	0.030025	0.056602	**0.047903**
Kif17	0.00971	0.004401	**0.011465**

Median value of the four gene expressions between the experimental and control groups in mature rats.
Statistically significant p values are shown in bold.

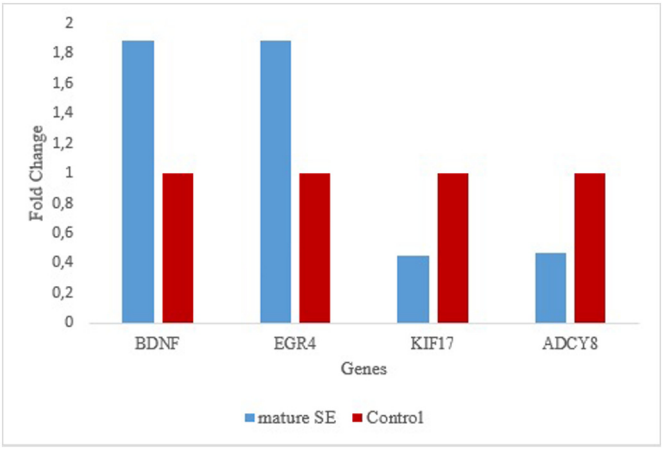


Fig. 3. Fold changes in mature SE. Fold changes of four gene expressions between the experimental and control groups in mature rats.

between initial dose and first dose of repeated injections was 10 min; then, subsequent doses were repeated by 5-min intervals.

Following the PTZ injection, the rats were placed to a plastic cage (50 × 20 × 25 cm in size), and seizure scoring was performed for 30 min [20]: stage 0, no response; stage 1, jerks at ear and face; stage 2, convulsion spreading to body; stage 3, myoclonic jerks or ramping on hind limbs; stage 4, clonic seizures with falling; 5, repeated tonic–clonic seizures or death.

The SE was defined as a single seizure lasting for at least 30 min or repeated myoclonic-clonic seizures by maximum of 5-min intervals for at least 30 min after the onset of the first stage 4 or 5 seizure. No intervention was made to discontinue the prolonged seizures. After spontaneous resolution of seizures, immature experiment and control groups were observed until weaning (day 22), while mature experiment and control groups were observed until completion of behavioral testing (day 72).

2.3. Behavior testing

2.3.1. Morris water maze

Morris water maze is a tool preferred to study spatial memory and learning in rodents such as rats. In our study, MWM (131 cm in diameter, 44 cm in depth) was used to test spatial learning in rats (day 72). The swimming speed, time in the target quadrant, escape time, and path length were recorded by “Ethovision” software (NOLDUS).

2.3.2. Open-field area

The rats were placed to the center of a square-shaped Plexiglas sheet (100 × 100 cm) which was divided into 16 equal squares. In the test, numbers of rearing, grooming, freezing, defecation, and lines crossed were noted during 5-min test period, and time at periphery and center of Plexiglas sheet were estimated by using video records. The Plexiglas sheet was cleaned by using 70% alcohol after each run.

Freezing was defined as lack of movement (except breathing) for at least 8 s. Rearing was defined as rearing up to hind limbs for at least 3 s. Grooming was defined as sweeping extremities and body for at least 10 s.

2.4. Preparation and assessment of hippocampal tissue samples

To harvest the hippocampus, the immature rats in the experiment and controls groups were killed on day 22 before behavioral testing while the mature rats in the mature experiment and control groups were killed on day 72 after behavioral testing by cervical dislocation at Genome and Stem Cell Center (GENKOK). The mRNA (messenger ribonucleic acid) gene expressions were assessed to study synaptic plasticity at molecular level.

2.4.1. Gene expression studies with quantitative real-time PCR

The RNA (ribonucleic acid) of the harvested hippocampal tissues was isolated by using Qiazol Lysis Buffer (Qiagen, Texas, USA) in accordance with manufacturer's instructions. Finally, the quantity (absorbance at 260 nm) and quality (ratio of absorbances at 260 nm and 280 nm) of RNA were evaluated with a BioSpec-Nano spectrophotometer. RNA was stored at −80 °C until used. First-strand complementary DNA (deoxyribonucleic acid) was synthesized from the total RNA with RT^2 First Strand Kit (Qiagen, Texas, USA). The experiment was conducted according to manufacturer's instructions. Qiagen RT^2 SYBR Green qPCR Mastermix Kit (Qiagen, Texas, USA) and commercially available RT^2 Profiler PCR Array (96-well) rat synaptic plasticity (Cat. No. 330231 PARN-126ZA) were used for real-time PCR. RT^2 Profiler DNA (deoxyribonucleic acid) Array (96-well) contains 84 genes (Table 1) and 6 housekeeping genes including Actb, B2m, Hprt1, Ldha, and Rplp1. The PCR program was run using Roche LightCycler® 480 II Real Time PCR. The changes in gene expression between the experiment and control groups were determined by the $2^{-\Delta\Delta Ct}$ method of relative quantification. Target gene copy numbers were normalized using whole housekeeping genes.

2.5. Statistical analysis

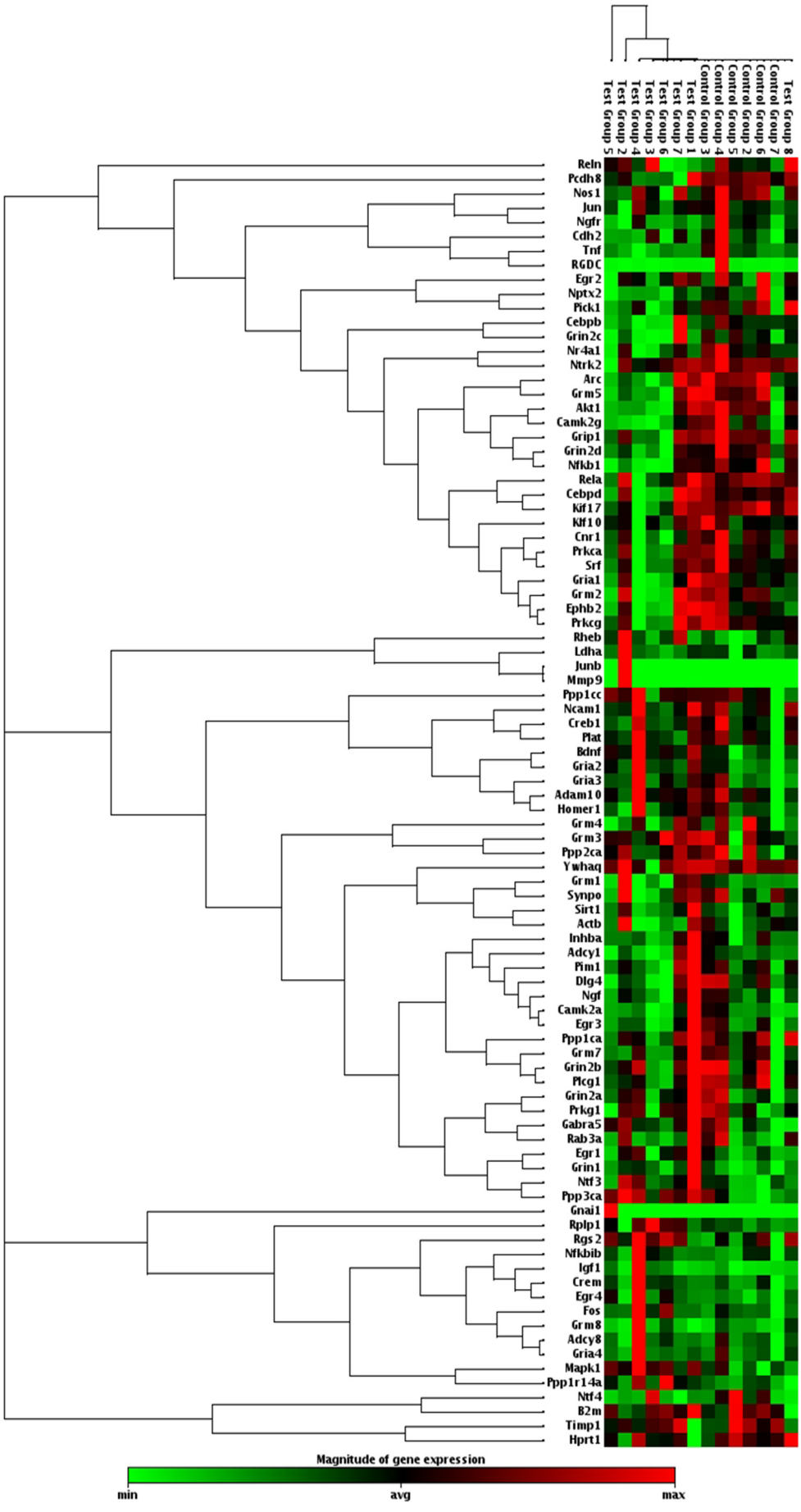
The normal distribution of the data was evaluated by histogram, q-q graphs, and Shapiro–Wilk test. Homogeneity of variance was evaluated by Levene's test. One-way analysis of variance (ANOVA) was used to compare open-field area parameters within groups. One-way ANOVA was used to compare MWM parameters within groups, while repeated measures ANOVA was used for comparisons between groups. Two samples and Wilcoxon's tests were performed to compare the differences between mRNA gene expression results between the groups. The data were analyzed using the SPSS 22.0 software. p values at 5% were considered significant.

3. Results

3.1. Comparison of weight, seizure latency, and number of injections

No significant difference was detected in the body weights between the rats in the experiment and control groups before and after SE (p >

Fig. 4. Heat map displaying the expression value of genes associated with synaptic plasticity in each group. Patterns of gene expression profiles for the 84 differentially expressed genes. MAPK1, PP3CA, and RSG2 gene expressions were determined significantly upregulated. TNF, ARC, and NR4A1 gene expressions were determined significantly downregulated in immature rats according to control groups ($p < 0.05$). In the cluster figure, columns represent samples, and row represents genes (black, red, and green correspond to unchanged, downregulated, and upregulated, respectively). Test group, hippocampus tissue of immature SE rats; control group, hippocampus tissue of control group. Test group: immature SE group, control group: immature control group.


Test Group 5
Test Group 2
Test Group 4
Test Group 3
Test Group 6
Test Group 7
Test Group 1
Control Group 3
Control Group 4
Control Group 5
Control Group 2
Control Group 6
Control Group 7
Test Group 8
Reln
Pcdh8
Nos1
Jun
Ngfr
Cdh2
Tnf
RGDC
Egr2
Nptx2
Pick1
Cebpb
Grin2c
Nr4a1
Ntrk2
Arc
Grm5
Akt1
Camk2g
Grip1
Grin2d
Nfkb1
Rela
Cebpd
Kif17
Klf10
Cnr1
Prkca
Srf
Gria1
Grm2
Ephb2
Prkcg
Rheb
Ldha
Junb
Mmp9
Ppp1cc
Ncam1
Creb1
Plat
Bdnf
Gria2
Gria3
Adam10
Homer1
Grm4
Grm3
Ppp2ca
Ywhaq
Grm1
Synpo
Sirt1
Actb
Inhba
Adcy1
Pim1
Dlg4
Ngf
Camk2a
Egr3
Ppp1ca
Grm7
Grin2b
Plcg1
Grin2a
Prkg1
Gabra5
Rab3a
Egr1
Grin1
Ntf3
Ppp3ca
Gnai1
Rplp1
Rgs2
Nfkbib
Igf1
Crem
Egr4
Fos
Grm8
Adcy8
Gria4
Mapk1
Ppp1r14a
Ntf4
B2m
Timp1
Hprt1
Magnitude of gene expression
min
avg
max

0.05) (Table 2). No difference was detected in time to SE (latency) and number of injections between the animals of the two experimental groups ($p > 0.05$). In all groups, SE lasted 30–120 min ($p > 0.05$), and no intervention was made to discontinue the seizures. Three rats died during SE episode; thus, they were excluded from the study.

3.2. Comparison of behavioral parameters

Behavioral parameters were assessed in the mature experiment group and the control group (Table 3). No significant difference was detected in any of the parameters in open-field area testing between the mature SE and control groups ($p > 0.05$).

3.3. Spatial memory and learning performance

In MWM test, there was no significant difference according to swim speed, spent time in the platform quadrant, escape time from the platform, and total distance between the mature SE and control groups ($p > 0.05$) (Tables 4a–4d).

3.4. Assessment of synaptic plasticity

3.4.1. Synaptic plasticity in immature rats

When fold changes were compared between the immature SE and control groups, six mRNA gene expressions were determined to be statistically significant according to the control group ($p < 0.05$). When compared according to fold change of mRNA gene expression, TNF (tumor necrosis factor), ARC (activity-regulated cytoskeleton-associated protein), and NR4A1 (nuclear receptor subfamily 4 group A member 1) gene fold changes were found less than those of the control group ($p = 0.032034$, $p = 0.03914$, and $p = 0{,}026212$, respectively). Whereas, BDNF, MAPK1 (mitogen-activated protein kinase 1), PPP3CA (protein phosphatase 3 catalytic subunit alpha), and RGS2 (regulator of G protein signaling 2) fold changes were found more than those of the control group ($p = 0.035638$, $p = 0.026212$, $p = 0.026212$, and $p = 0.028457$, respectively) (Table 5 and Fig. 2).

3.4.2. Synaptic plasticity in mature rats

When fold changes were compared between the mature SE and control groups for mRNA gene expression, a statistically significant difference was determined compared to the control group. When the fold changes of mRNA gene expression were compared, the BDNF and EGR4 (early growth response 4) gene fold changes were found to be more than those of the control group ($p = 0.02911$, $p = 0.047903$, respectively). Whereas, KIF17 (kinesin family member 17) and ADCY8 (adenylate cyclase 8) fold changes were found to be less than those of the control group ($p = 0.011465$, $p = 0.01252$, respectively) (Table 6 and Fig. 3). Figs. 4 and 5 show the cluster diagram analysis of synaptic plasticity-related genes.

4. Discussion

Defining the changes in molecular mechanisms of synaptic plasticity pathways triggered by seizures will help to understand the harmful effects of seizures on neuronal networks and cognitive functions [18]. After the SE, memory, learning, and behavior may be impaired [21]. The progress and spread of seizures, EEG characteristics, behavioral characteristics, and the consequences of seizures are associated with brain maturation [22]. Therefore, in this study, the differences in the expressions of the 84 genes that play a key role in synaptic plasticity after PTZ-induced SE in the mature and immature brain were determined, and the effects of SE on memory, learning, and behaviors were investigated.

Animal studies have shown that long-lasting seizures may cause cognitive dysfunction [23]. Synaptic plasticity is one of the most important mechanisms in the formation of cognitive functions [24]. Electrophysiological studies suggest that short- and long-term synaptic plasticity in cortex and hippocampus may vary significantly after seizures [25,26]. However, because of the contradictory results of experimental studies, the pattern and main mechanisms of these changes are still unclear. In this study, we evaluated the effect of SE on the changes of synaptic plasticity gene expressions which are responsible for LTP and LTD formations.

Immature rats and emotional or learning problems were not reported [27,28]. In our study, there was no difference between the mature and control groups in the open-field and MWM behavior, memory, and learning after the SE, which was evaluated as compatible with the literature findings.

Before this, there is no study performed concerning effects of PTZ-induced SE. In our study, after PTZ-induced SE, differences were observed in the gene expression of ADCY8, BDNF, PPP3CA, and MAPK1 genes which are LTP-related genes, whereas differences were observed in PPP3CA and MAPK1 gene expressions which are LTD-related genes.

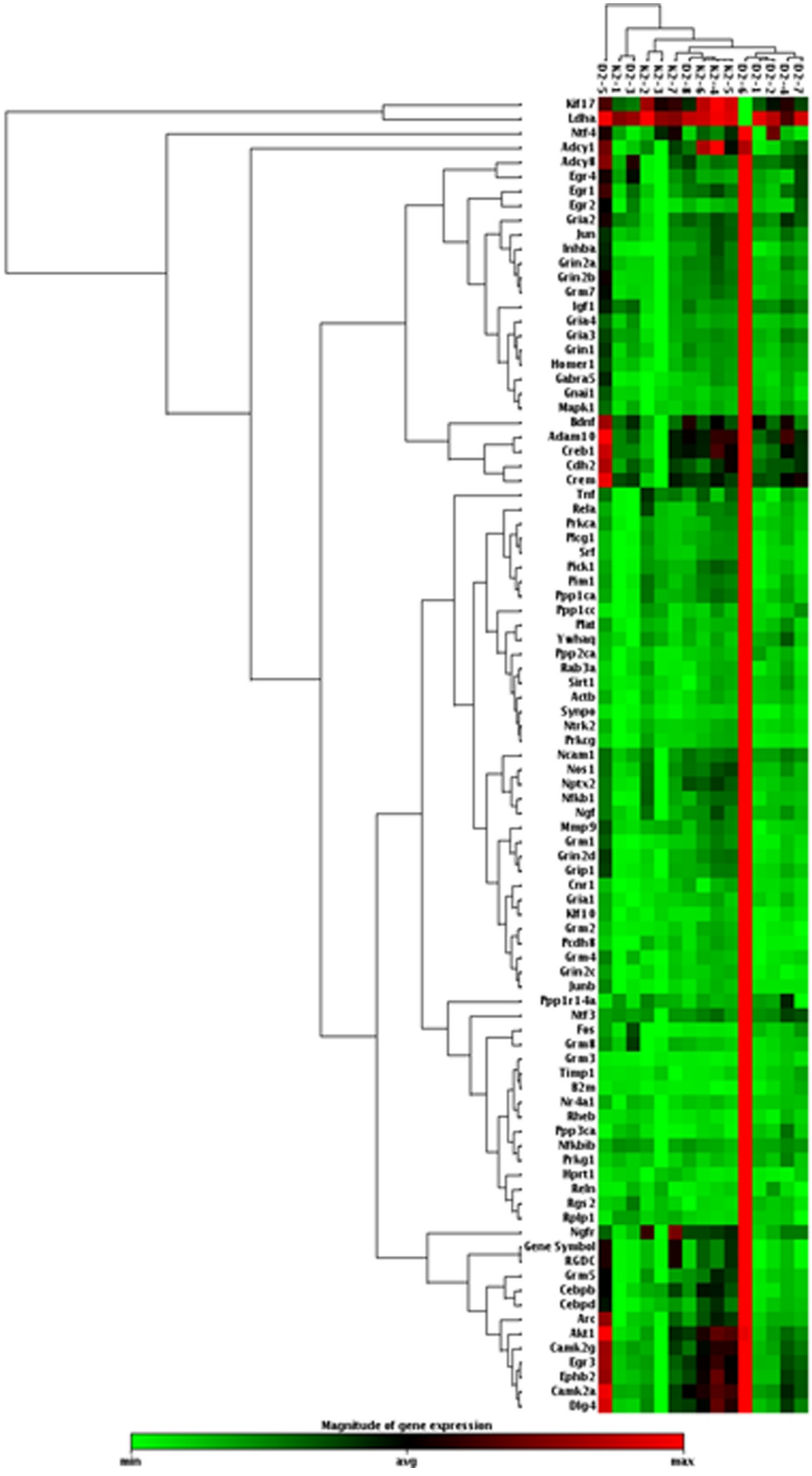
It is known that MAPK1 plays an important role in cellular transmission in NMDA (N-methyl-D-aspartate) receptors [29]. The significant increase in MAPK1 expression in the immature SE group in our study supports the idea that MAPK1 plays a role in the disruption of synaptic plasticity, especially associated with LTP.

In our the study, the significant increase in ADCY8 mRNA gene expression in the mature SE group suggests that ADCY8 may play a role at the molecular level in epileptogenesis and that the downstream flow stream (Cyclic adenosine monophosphate (cAMP)-Extracellular signal-regulated kinase (ERK)1/2-cAMP-responsive element-binding protein (CREB) pathway) may be a potential target in epileptic treatment. In a study, the importance of ADCY8 in LTP and synaptic plasticity was emphasized, and it was stated that it played a role in the consolidation of memory and long-term memory related to fear [30]. In another study, ADCY8 inactive mice showed a decrease in susceptibility to kainic acid and pilocarpine from chemical convulsions [31]. In addition, cAMP and cAMP-dependent protein kinase A is involved in epileptiform discharge in rat hippocampus [32].

In our study, there was a significant increase in BDNF mRNA expression in mature and immature experimental groups after PTZ-induced SE. Brain-derived neurotrophic factor mRNA and protein are significantly upregulated in the hippocampus by seizure activity in animal models [33], and infusion of anti-BDNF agents [34], or BDNF knockout [35] or truncated tropomyosin-related kinase B (trkB) overexpressing [36] mice inhibits epileptogenesis in animal models. Conversely, direct application of BDNF induces hyperexcitability in vitro [37]; overexpression of BDNF in transgenic mice leads to spontaneous seizures [38]; and intrahippocampal infusion of BDNF is sufficient to induce seizure activity in vivo [39]. The hippocampus and closely related limbic structures are thought to be particularly important in the proepileptogenic effects of BDNF [34], and in fact, increased BDNF expression in the hippocampus is found in specimens from patients with temporal lobe epilepsy [40]. Understanding the hyperexcitability to BDNF in animal models of epilepsy is promising for new anticonvulsant or antiepileptogenic therapies [41].

As it is known, exposure to seizure is the basis for the development of new seizures, the data we obtained in our study support the

Fig. 5. Heat map displaying the expression value of genes associated with synaptic plasticity in each group. Patterns of gene expression profiles for the 84 differentially expressed genes. BDNF and EGR4 gene expressions were determined significantly upregulated. KIF17 and ADCY8 gene expressions were determined significantly downregulated in immature rats according to control groups ($p < 0.05$). In the cluster figure, columns represent samples, and row represents genes (black, red, and green correspond to unchanged, downregulated, and upregulated, respectively). Test group, hippocampus tissue of mature SE rats; control group, hippocampus tissue of control group. D2: mature SE group, K2: mature control group.


Gene Symbol
Magnitude of gene expression
min
avg
max

knowledge that BDNF has proepileptic effects. In addition, the significant increase in BDNF mRNA gene expression in the SE experimental groups raises the question of whether we can determine the level of BDNF mRNA in serum and determine progression in epilepsy using a practical method that can be applied in clinical trials [42].

In our study, increased mRNA expression of PPP3CA genes and decreased ARC gene mRNA expression were found in immature SE group. In addition, increased expression of EGR4 in the mature experimental group was observed. Protein phosphatase 3 catalytic subunit alpha gene functions in the regulation of neuronal cell skeletal structure and on synaptic plasticity still remain unclear [43]. In our study, ARC and EGR4 gene expression changes were consistent with the literature.

Expression of EGR4 gene-associated plasticity was found to be increased, and synaptic conductivity was found to be defective [44]. In a previous study, schizophrenia and the EGR4 gene were associated with male patients [45]. It is known that EGR4 functions as a transcriptional suppressor and displays autoregulation activities, and also regulates its gene expression that is downregulated depending on dose [46]. Activity-regulated cytoskeleton associated protein expression is required for long-term memory consolidation. However, it is not for learning or short-term memory formation [47].

In our study, we demonstrated that a significant decrease in Tnf-α mRNA gene expression in the immature SE group which could be explained by expected an inflammatory response is defective after SE, the clinical relevance of this finding needs further investigations.

In inflammatory epilepsies, significant inflammatory reactions in the epileptogenic area have been shown, which were considered to affect excitability. This inflammatory reaction in rats has been shown to increase neuronal excitability, lower seizure threshold, and prolonged seizure duration, and the seizures reduce with some antiinflammatory drugs [48]. According to the literature, inflammatory processes are dominant in drug-resistant epilepsies in terms of antiinflammatory process.

In physiological conditions, astrocytes play a central role in synaptic integration and neuronal processes. In particular, they cause the release of TNF-a from astrocytes and the simultaneous activation of various processes during extended synaptic activity [49]. The reduction of TNF-α mRNA gene expression in the immature SE group may be considered as an indication of impaired synaptogenesis when the above-mentioned processes, activated together with TNF-α release, were evaluated.

As there were no recurring seizures in our study, the experimental groups were not exposed to any chronic stress. This may be the reason why we could not find significant differences in behavioral tests and reduction of TNF-a in the immature SE group.

The NR4A (nuclear receptor subfamily 4 group A) receptors are associated with pathological inflammatory conditions (cancer, immunological changes, and metabolic, cardiovascular, or neurological diseases etc.) [50]. One study demonstrated that hippocampal overexpression of NR4A1 may improve age-related memory disorders [51]. Nuclear receptor subfamily 4 group A is induced in the mouse hippocampus by contextual fear conditioning or histone deacetylase inhibitors [52].

In our study, the decrease in NR4A1 mRNA gene expression in the immature SE group in the hippocampus may be helpful to explain to unexpected inflammatory conditions related to the reduction of TNF-a after epileptic seizures. Regarding the effects of NR4A1 on memory functions in the immature brain, there are no data in the literature. We concluded that long-lasting seizures may affect the emotional memory processes transiently in hippocampus during the immature period.

In our study, the significant decrease in KIF17 expression in the immature experimental group supports the fact that postseizure KIF17 is one of the molecular mechanisms involved in learning and memory after PTZ-induced SE. It has been found that overexpression of KIF17 increases spatial and working memory in transgenic mice [53], and it was found that performance in learning and memory tests was impaired in recent function loss studies in KIF17 −/− mice [54]. The critical role of KIF17 in transporting the NR2B (N-methyl D-aspartate receptor subtype 2B) subunit to postsynaptic dendrites [55], and the neuronal plasticity of NR2B plays a key role in learning and memory [56]. Taken into account, in the literature data, we concluded that, although the molecular mechanism of memory and learning was changed, we could not find any clue using behavioral tests regarding these effects of PTZ-induced SE.

Considering the fact that RGSs (regulator of G protein signaling) regulate the G protein-mediated signal kinetics and affect the specificity of signal transduction, it is no surprise that we detected a significant increase in the expression of RGS2, which acts as a negative signal regulator by decreasing the G protein signal duration, in immature SE group [57].

Activity-regulated cytoskeleton-associated protein, BDNF, MAPK1, NR4A1, PPP3CA, RGS2, TNF mRNA gene expressions were different in the immature SE group; whereas, ADCY8, BDNF, EGR4, KIF17 mRNA gene expressions were different in the mature SE group. The most striking is the increased expression of the BDNF gene in both immature SE and mature SE groups. This observation may indicate that BDNF has a more important role than other genes in SE. In the immature SE group, the fact that there were no significant differences in gene expression in ARC, MAPK1, NR4A1, PPP3CA, RGS2, TNF genes can be explained by the absence of recurrent seizures. Recurrent seizures may lead to persistence of changes in gene expression in the mature period. Expression changes in the ADCY8, EGR4, KIF17 genes that were detected only in the mature SE group lead us to a question, could this cause of gene expression changes be the late-term effect of SE?

There are no biomarkers for epileptogenesis. The relationship of inflammation with epilepsy seizures is one of the subjects that attract attention in recent years. It has been shown in animal studies that the synthesis of various cytokines and adhesion molecules has increased [48]. Only levels of inflammatory proteins in circulating blood have been proposed as potential biomarkers of epileptogenesis [58]. In a human study, postictal and interictal cytokine levels were compared, and their levels were significantly higher in the postictal period [59]. When our findings are evaluated, it can be thought that results of gene expression may be potential biomarkers; however, new studies are needed to support this idea because epileptogenesis is a dynamic process and cannot be explained by a single mechanism.

5. Conclusions

In this study, we evaluated the expression of all synaptic plasticity genes after PTZ-induced SE in mature and immature rats for the first time. Although there was no significant difference in behavioral tests after SE induced by PTZ, a significant difference was observed in gene expression levels which play a role in learning memory and synaptic plasticity at the molecular studies. Although there is no impairment of learning memory after resistant seizures, the expression of genes that play important roles in learning memory is affected. It is not easy to explain epileptogenesis by a single mechanism. Comorbid conditions should also be considered in this respect. The role of synaptic plasticity in epileptogenesis is obvious, and the changes in gene expression following ES in our study support this information.

Considering our findings, it may be thought that gene expression products in our study may be potential biomarkers, but epileptogenesis is a dynamic process and cannot be explained by a single mechanism. Future studies on epileptogenesis, and studies that will be designed specifically on genes in which we detected differences in expressions will elucidate better the synaptic plasticity and epileptogenesis in epilepsy.

Acknowledgments

This project was supported by Erciyes University Scientific Research Projects Coordination Unit with the code TTU-2016-6642. There are no conflicts of interest to declare.

References

[1] Shovron S. Status epilepticus: its clinical features and treatment in adults and children. Cambridge: Cambridge University Press; 1994; 21–6.
[2] Macdonald RL, Kapur J. Acute cellular alterations in the hippocampus after status epilepticus. Epilepsia 1999;40.
[3] Bora İ, Takapilioğlu Ö. New horizons in epilepsy treatment. J Turk Chap ILAE 2003;9:91–102.
[4] Ransom CB, Blumenfeld H. Acquired epilepsy: cellular and molecular mechanisms. Molecular neurology. Elsevier; 2007. p. 347–70.
[5] Contestabile A. Roles of NMDA receptor activity and nitric oxide production in brain development. Brain Res Rev 2000;32:476–509.
[6] Nelson CA, Thomas KM, De Haan M. Neuroscience of cognitive development: the role of experience and the developing brain. John Wiley & Sons; 2012; 25.
[7] Bennett M. The concept of long term potentiation of transmission at synapses. Prog Neurobiol 2000;60:109–37.
[8] Lomo T. The discovery of long-term potentiation. Philos Trans R Soc Lond B Biol Sci 2003;358:617–20.
[9] Blitzer RD. Long-term potentiation: mechanisms of induction and maintenance. Sci Signal 2005;2005:tr26.
[10] Alkadhi K, Alzoubi K, Aleisa A. Plasticity of synaptic transmission in autonomic ganglia. Prog Neurobiol 2005;75:83–108.
[11] Lisman J, Spruston N. Postsynaptic depolarization requirements for LTP and LTD: a critique of spike timing-dependent plasticity. Nat Neurosci 2005;8:839.
[12] Blitzer RD, Iyengar R, Landau EM. Postsynaptic signaling networks: cellular cogwheels underlying long-term plasticity. Biol Psychiatry 2005;57:113–9.
[13] Bruel-Jungerman E, Davis S, Rampon C, Laroche S. Long-term potentiation enhances neurogenesis in the adult dentate gyrus. J Neurosci 2006;26:5888–93.
[14] Schmidt-Hieber C, Jonas P, Bischofberger J. Enhanced synaptic plasticity in newly generated granule cells of the adult hippocampus. Nature 2004;429:184.
[15] Aicardi G, Argilli E, Cappello S, Santi S, Riccio M, Thoenen H, et al. Induction of long-term potentiation and depression is reflected by corresponding changes in secretion of endogenous brain-derived neurotrophic factor. Proc Natl Acad Sci 2004;101:15788–92.
[16] Minatohara K, Akiyoshi M, Okuno H. Role of immediate-early genes in synaptic plasticity and neuronal ensembles underlying the memory trace. Front Mol Neurosci 2016;8:78.
[17] Vingerhoets G, Deblaere K, Backes WH, Achten E, Boon P, Boon PJ, et al. Lessons for neuropsychology from functional MRI in patients with epilepsy. Epilepsy Behav 2004;5:81–9.
[18] Meldrum BS, Bruton CJ. Epilepsy. In: Adams JH, Duchen LW, editors. Greenfield's neuropathology. New York: Oxford University Press; 1992. p. 1246–83.
[19] Najm I, Janigro D, Babb T. Mechanisms of epileptogenesis and experimental models of seizures. The treatment of epilepsy: principles and practice, vol. 3. ; 2001. p. 33–4.
[20] Lamberty Y, Klitgaard H. Consequences of pentylenetetrazole kindling on spatial memory and emotional responding in the rat. Epilepsy Behav 2000;1:256–61.
[21] Rice AC, Floyd CL, Lyeth BG, Hamm RJ, DeLorenzo RJ. Status epilepticus causes long-term NMDA receptor-dependent behavioral changes and cognitive deficits. Epilepsia 1998;39:1148–57.
[22] Holmes GL. Epilepsy in the developing brain: lessons from the laboratory and clinic. Epilepsia 1997;38:12–30.
[23] Aniol VA, Ivanova-Dyatlova AY, Keren O, Guekht AB, Sarne Y, Gulyaeva NV. A single pentylenetetrazole-induced clonic-tonic seizure episode is accompanied by a slowly developing cognitive decline in rats. Epilepsy Behav 2013;26:196–202.
[24] Kandel ER. The molecular biology of memory storage: a dialog between genes and synapses. Biosci Rep 2004;24:475–522.
[25] Müller L, Tokay T, Porath K, Köhling R, Kirschstein T. Enhanced NMDA receptor-dependent LTP in the epileptic CA1 area via upregulation of NR2B. Neurobiol Dis 2013;54:183–93.
[26] Zhou JL, Shatskikh TN, Liu X, Holmes GL. Impaired single cell firing and long-term potentiation parallels memory impairment following recurrent seizures. Eur J Neurosci 2007;25:3667–77.
[27] Kubová H, Mareš P, Suchomelová L, Brožek G, Druga R, Pitkänen A. Status epilepticus in immature rats leads to behavioural and cognitive impairment and epileptogenesis. Eur J Neurosci 2004;19:3255–65.
[28] Erdoğan F, Gölgeli A, Küçük A, Arman F, Karaman Y, Ersoy A. Effects of pentylenetetrazole-induced status epilepticus on behavior, emotional memory and learning in immature rats. Epilepsy Behav 2005;6:537–42.
[29] Goldsmith Z, Dhanasekaran D. G protein regulation of MAPK networks. Oncogene 2007;26:3122.
[30] Wolf EJ, Rasmusson AM, Mitchell KS, Logue MW, Baldwin CT, Miller MW. A genome-wide association study of clinical symptoms of dissociation in a trauma-exposed sample. Depress Anxiety 2014;31:352–60.
[31] Chen X, Dong G, Zheng C, Wang H, Yun W, Zhou X. A reduced susceptibility to chemoconvulsant stimulation in adenylyl cyclase 8 knockout mice. Epilepsy Res 2016;119:24–9.
[32] Higashima M, Ohno K, Koshino Y. Cyclic AMP-mediated modulation of epileptiform after discharge generation in rat hippocampal slices. Brain Res 2002;949:157–61.
[33] Nibuya M, Morinobu S, Duman RS. Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. J Neurosci 1995;15:7539–47.
[34] Binder DK, Routbort MJ, Ryan TE, Yancopoulos GD, McNamara JO. Selective inhibition of kindling development by intraventricular administration of TrkB receptor body. J Neurosci 1999;19:1424–36.
[35] Kokaia M, Ernfors P, Kokaia Z, Elmér E, Jaenisch R, Lindvall O. Suppressed epileptogenesis in BDNF mutant mice. Exp Neurol 1995;133:215–24.
[36] Lähteinen S, Pitkänen A, Saarelainen T, Nissinen J, Koponen E, Castrén E. Decreased BDNF signalling in transgenic mice reduces epileptogenesis. Eur J Neurosci 2002;15:721–34.
[37] Scharfman HE. Hyperexcitability in combined entorhinal/hippocampal slices of adult rat after exposure to brain-derived neurotrophic factor. J Neurophysiol 1997;78:1082–95.
[38] Croll S, Suri C, Compton D, Simmons M, Yancopoulos G, Lindsay R, et al. Brain-derived neurotrophic factor transgenic mice exhibit passive avoidance deficits, increased seizure severity and in vitro hyperexcitability in the hippocampus and entorhinal cortex. Neuroscience 1999;93:1491–506.
[39] Scharfman HE, Goodman JH, Sollas AL, Croll SD. Spontaneous limbic seizures after intrahippocampal infusion of brain-derived neurotrophic factor. Exp Neurol 2002;174:201–14.
[40] Mathern GW, Babb TL, Micevych PE, Blanco CE, Pretorius JK. Granule cell mRNA levels for BDNF, NGF, and NT-3 correlate with neuron losses or supragranular mossy fiber sprouting in the chronically damaged and epileptic human hippocampus. Mol Chem Neuropathol 1997;30:53–76.
[41] Binder DK, Croll SD, Gall CM, Scharfman HE. BDNF and epilepsy: too much of a good thing? Trends Neurosci 2001;24:47–53.
[42] Hensch TK. The power of the infant brain. Sci Am 2016;314:64–9.
[43] Hoffman A, Taleski G, Sontag E. The protein serine/threonine phosphatases PP2A, PP1 and calcineurin: a triple threat in the regulation of the neuronal cytoskeleton. Mol Cell Neurosci 2017;84:119–31.
[44] Wang H-Y, Hsieh P-F, Huang D-F, Chin P-S, Chou C-H, Tung C-C, et al. RBFOX3/NeuN is required for hippocampal circuit balance and function. Sci Rep 2015;5:17383.
[45] Cheng MC, Chuang YA, Lu CL, Chen YJ, Luu SU, Li JM, et al. Genetic and functional analyses of early growth response (EGR) family genes in schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry 2012;39:149–55.
[46] Zipfel PF, Decker EL, Holst C, Skerka C. The human zinc finger protein EGR-4 acts as autoregulatory transcriptional repressor. Biochim Biophys Acta 1997;1354:134–44.
[47] Plath N, Ohana O, Dammermann B, Errington ML, Schmitz D, Gross C, et al. Arc/Arg3.1 is essential for the consolidation of synaptic plasticity and memories. Neuron 2006;52:437–44.
[48] Vezzani A, Granata T. Brain inflammation in epilepsy: experimental and clinical evidence. Epilepsia 2005;46:1724–43.
[49] McAfoose J, Baune B. Evidence for a cytokine model of cognitive function. Neurosci Biobehav Rev 2009;33:355–66.
[50] Zhang L, Wang Q, Liu W, Liu F, Ji A, Li Y. The orphan nuclear receptor 4A1: a potential new therapeutic target for metabolic diseases. J Diabetes Res 2018;2018.
[51] Kwapis JL, Alaghband Y, López AJ, Long JM, Li X, Shu G, et al. HDAC3-mediated repression of the Nr4a family contributes to age-related impairments in long-term memory. J Neurosci 2019:2799-18.
[52] Hawk JD, Bookout AL, Poplawski SG, Bridi M, Rao AJ, Sulewski ME, et al. NR4A nuclear receptors support memory enhancement by histone deacetylase inhibitors. J Clin Invest 2012;122:3593–602.
[53] Wong RW, Setou M, Teng J, Takei Y, Hirokawa N. Overexpression of motor protein KIF17 enhances spatial and working memory in transgenic mice. Proc Natl Acad Sci U S A 2002;99:14500–5.
[54] Yin X, Takei Y, Kido MA, Hirokawa N. Molecular motor KIF17 is fundamental for memory and learning via differential support of synaptic NR2A/2B levels. Neuron 2011;70:310–25.
[55] Setou M, Nakagawa T, Seog DH, Hirokawa N. Kinesin superfamily motor protein KIF17 and mLin-10 in NMDA receptor-containing vesicle transport. Science 2000;288:1796–802.
[56] Bannerman DM. Fractionating spatial memory with glutamate receptor subunit-knockout mice. Biochem Soc Trans 2009;37:1323–7.
[57] Hollinger S, Hepler JR. Cellular regulation of RGS proteins: modulators and integrators of G protein signaling. Pharmacol Rev 2002;54:527–59.
[58] Lukasiuk K, Becker AJ. Molecular biomarkers of epileptogenesis. Neurotherapeutics 2014;11:319–23.
[59] Sinha S, Patil SA, Jayalekshmy V, Satishchandra P. Do cytokines have any role in epilepsy? Epilepsy Res 2008;82:171–6.